Pergamon

Tetrahedron Letters 39 (1998) 1243–1246

TETRAHEDRON LETTERS

Site-Specific *N*-Alkylation of Peptides on the Solid Phase

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Received 23 October 1997; revised 3 December 1997; accepted 5 December 1997

Abstract: A convenient approach, featuring a Mitsunobu reaction, is described for the introduction of an alkyl group at a specific amide function of a peptide on the solid phase. This 'site-specific alkylation' procedure is illustrated with an *N*-ethyl scan of Leu-enkephalin.

Applications of peptides as drugs are often hampered by poor bioavailability because of their water-solubility and biodegradation by *e.g.* proteases.[1] At least a partial remedy of these disadvantages, while retaining the biological activity of a peptide, can be achieved by the introduction of modified amino acids or by using peptidomimetics.[2] A particularly interesting modification is *N*-alkylation. In fact a very interesting class of peptidomimetics comprises the peptoids,[3] which consist of *N*-alkylated glycine residues. They possess an increased metabolic stability[4] as well as an increased lipophilicity. However, it is usually unknown to what extent *N*-alkylation of a peptide will interfere with the biological activity. In order to be able to evaluate this, our objective is to develop a convenient method for the introduction of *N*-alkyl groups onto (a) specific amide function(s) within a peptide in an approach denoted as "site-specific *N*-alkylation".

N-alkylated glycine derivatives can be obtained in straightforward ways,[3,5] whereas the preparation of *N*-alkylated amino acids other then glycine is certainly not trivial. The method of Seebach *et al.*[6] is very attractive for non-selective *N*-peralkylation of peptide-amide bonds and the only recently published selective *N*-alkylation method is "site-selective methylation".[7] This prompted us to communicate our general procedure for site-specific *N*-alkylation of peptides on the solid phase, using a Mitsunobu reaction. As an illustration of the versatility of the method we have performed an *N*-ethyl scan of Leu-enkephalin.

Several methods have been developed for the *N*-alkylation of amino acids. The well-known method of reductive amination in solution[8,9b] or on the solid phase[9] suffers *e.g.* from dialkylation.[9b] An interesting approach is the alkylation of sulfonamides using *e.g.* the PMC[10] or Ts[11] protecting groups. Both groups can only be removed under harsh conditions and are therefore not suitable for solid phase synthesis.

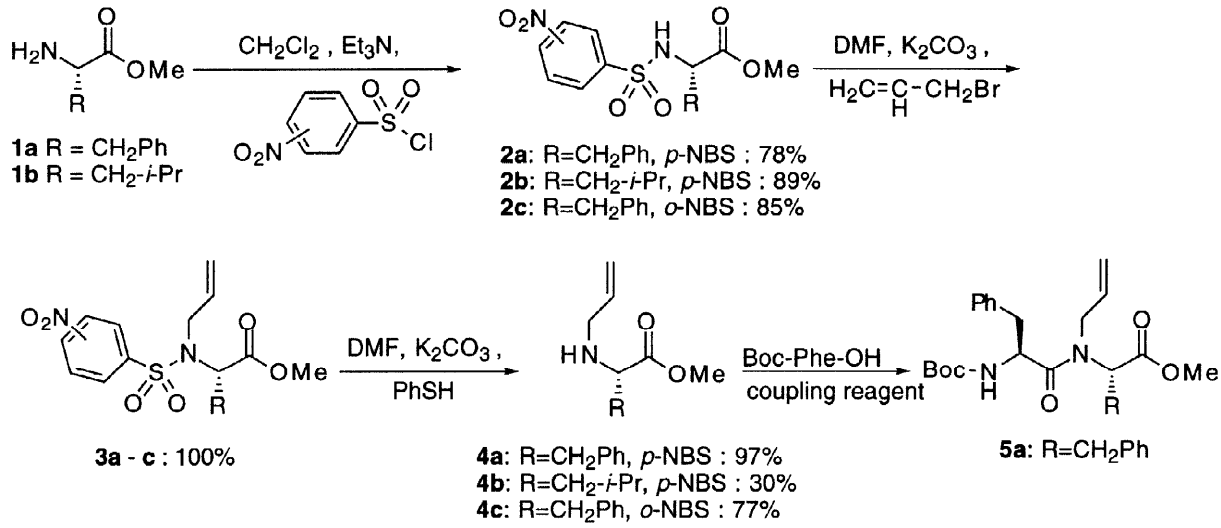
For a variety of reasons we are interested in the synthesis of *N*-allyl substituted amino acids as model compounds. Clearly, we started by developing a general method for synthesis in solution with the aim to extend this to both a high yielding and clean solid phase synthesis. In order to realize this we applied the method of Fukuyama[12] for the preparation of secondary amines to the synthesis of *N*-allyl-substituted amino acids (Scheme 1). The *p*-nitro-benzenesulfonyl-group was introduced as an amino protective group by reaction of *p*-nitro-benzenesulfonchloride with the methyl ester of amino acids **1** to give sulfonamides **2**. The increased acidity of the sulfonamide N-H allowed alkylation using allylbromide in the presence of potassium carbonate and *N*-allyl-sulfonamides **3** were obtained in quantitative yields. Deprotection of these *N*-allyl-sulfonamides by treatment with thiophenol in the presence of potassium carbonate led to *N*-allyl-amino acids **4a** and **4b** in 97% and 30%, respectively.[13]

The coupling of *N*-allyl-amino acids **4** to dipeptides **5** turned out to be quite difficult. Several coupling reagents were evaluated. The more commonly used coupling reagents: BOP, PyBroP, DIC and TFFH, gave very poor results (<10%). The coupling reagents based on chloro-formamidinium *viz.* TClFH, CIP and PyClU

0040-4039/98/$19.00
PII: S0040-4039(97)10766-3

as well as BOP-Cl, gave moderate results (~30%). Only the coupling reagents based on HOAt *viz.* PyBroP/HOAt and HATU,[14] gave acceptable yields (>60%), using 2 equivalents and coupling overnight.

Despite the occasionally modest yields of the synthesis in solution, we felt we had to translate the solution procedure to a solid phase method, since our main objective was to develop a mild method for site-specific *N*-alkylation **on the solid phase**. We planned to use plain Tentagel-OH® resin, since it would be particularly advantageous to have a mild method which does not require additional expensive linkers.

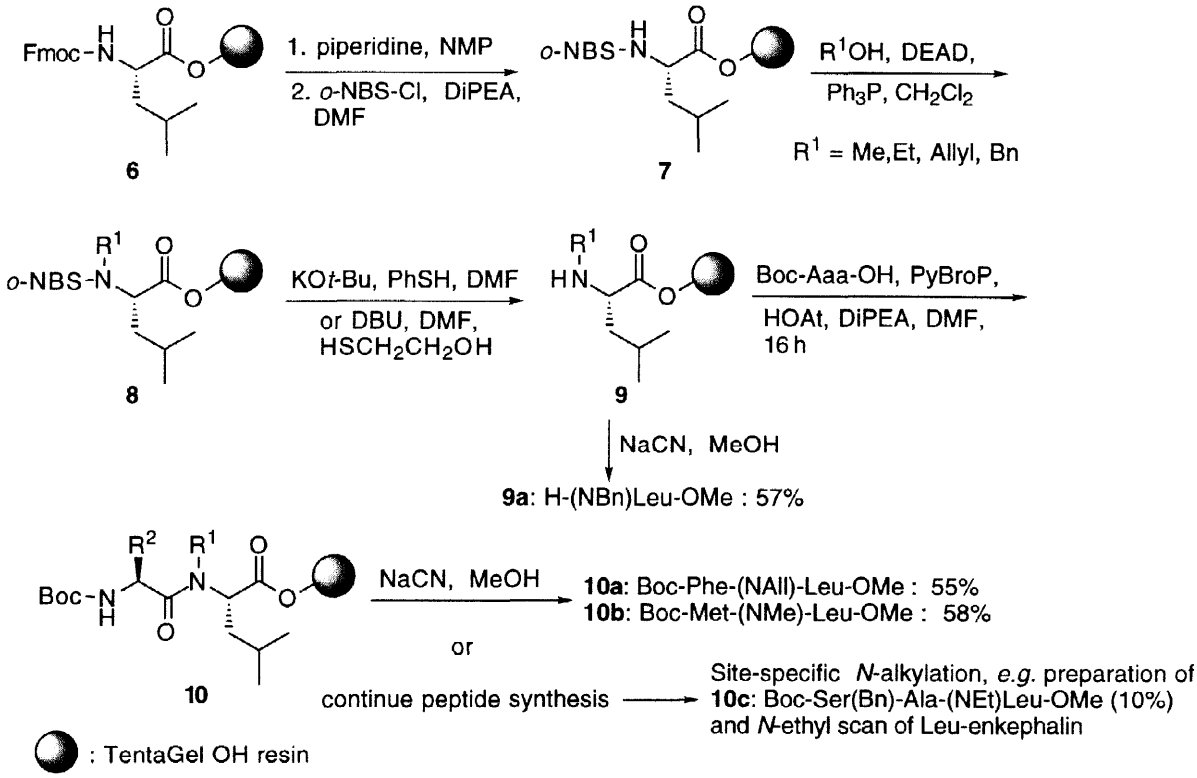


Scheme 1.

To this end, a Fmoc protected amino acid was coupled to Tentagel-OH® according to the method of Sieber.[15] After removal of the Fmoc-group in **6**, the *o*-nitrobenzenesulfonamide group instead of the earlier used *p*-nitrobenzenesulfonamide group was introduced (Scheme 2).[16] In subsequent steps, especially the deprotection step, the use of the *o*-nitrobenzenesulfonamide (*o*-NBS) group turned out to give better results than *p*-nitrobenzenesulfonamide and therefore use of the former was preferred (see also Scheme 1, **4c**). For alkylation of the sulfonamide **7** the Mitsunobu reaction was chosen using DEAD and the necessary alcohol, because this reaction is far more convenient on the solid phase than in solution and moreover, it can be performed in the presence of amides (*vide infra*). Furthermore, a large diversity of alcohols is commercially available, which enables the preparation of a variety of alkylated peptides. Finally, the Mitsunobu reaction conditions are such that removal of the modified peptide from the resin by saponification and racemization are very unlikely. Application of the conditions of the solution synthesis (Scheme 1) *i.e.* K_2CO_3 and thiophenol in DMF, to removal of the *o*-NBS-group of **8**, resulted partly in saponification. Using KO*t*-Bu as a base or 2-mercapto-ethanol with DBU,[7] immediately followed by acidification by quenching with 25% HOAc in DMF to prevent saponification, was more successful. The use of DBU or DiPEA as base in combination with thiophenol gave poor results but DBU in combination with 2-mercapto-ethanol gave slightly better yields of **9** than KO*t*-Bu with thiophenol. Coupling of this *N*-alkylated amino acid **9** with a second amino acid to afford **10** was achieved by using 3 equivalents of PyBroP, HOAt and the Fmoc-amino acid in DMF. The use of HATU gave slightly lower yields. Also the use of other solvents, like DMA, MeCN and NMP, resulted in a slower reaction and low yields. A few di- and tripeptides *i.e.* **10a-c** were prepared in order to have an impression of the scope (Scheme 2). The introduction by the Mitsunobu reaction of different primary alcohols was performed without any problems, but coupling of the next amino acid became more difficult with increasing steric bulk of the alkyl group. The coupling reaction of the *N*-Me derivative, leading to **10b** gave slightly better results than that leading to the *N*-allyl derivative **10a**.

The synthesis of tripeptides is hampered by formation of diketopiperazines,[9] which occurs after removal of the Fmoc-group by base. Therefore, at the dipeptide stage it is necessary to use an acid labile protecting

group instead of the Fmoc group. For this purpose, we chose the Boc group and BOP as a coupling agent for introduction of the third amino acid. However, still significant reductions of the yields were found due to formation of a diketopiperazine structure, for example in the preparation of **10c**. Fortunately, it was possible to obtain better yields when HATU was used instead of BOP.



Scheme 2.

The site-specific approach is of particular interest in peptides larger than dipeptides, since it can be used *e.g.* to increase metabolic stability or to decrease conformational flexibility by backbone cyclization. In order to demonstrate this method we performed an *N*-Ethyl scan of Leu-enkephalin by a parallel solid-phase synthesis approach. Peptide synthesis was carried up to the position where *N*-ethylation was desired. Then, the Fmoc group was then removed and the resin bound peptide was alkylated at the amino terminus according to the protocol delineated above (Scheme 2). The next Fmoc amino acid was then activated by PyBroP/HOAt in DMF and the coupling was carried out overnight, followed by continuation of the standard Fmoc peptide synthesis. In order to facilitate purification a fully protected peptide was preferred and also the Fmoc group from tyrosine was replaced by a Boc group.

Table 1. *N*-Ethyl scan of Leu-Enkephalin.

N-alkylated peptide	isolated yield (%)	purity (%)[a]
Boc-Tyr(*t*-Bu)-Gly-Gly-Phe-(*N*-Et)Leu-OMe	43[b]	84
Boc-Tyr(*t*-Bu)-Gly-Gly-(*N*-Et)Phe-Leu-OMe	29	83
Boc-Tyr(*t*-Bu)-Gly-(*N*-Et)Gly-Phe-Leu-OMe	26	84
Boc-Tyr(*t*-Bu)-(*N*-Et)Gly-Gly-Phe-Leu-OMe	12	95

a) according to HPLC; b) contains 14% of a byproduct, probably: Boc-Tyr(*t*-Bu)-Gly-Gly-(*N*-Et)Leu-OMe

Cleavage from the resin was carried out using MeOH/NaCN and the resulting peptide methyl esters were purified by silica gel column chromatography. Introduction of the *o*-NBS-group at the glycine residue gave rise to side reactions, but these could be suppressed by using CH_2Cl_2 as a solvent instead of DMF. All peptides

were reasonably pure according HLPC (see table 1) and disappointingly the yields are still low to moderate most likely due to premature saponification. FAB mass spectrometry showed that the four synthesized *N*-ethylated peptides have identical masses and unambiguously established the site of ethylation in each peptide. The *N*-ethylated residue is readily identified by its prominent *N*-ethylimmonium ion peak and analysis of the peptide fragments in the mass spectra was used to confirm its exact location in the peptide sequence.

In conclusion, we have shown that amino acids can be alkylated in a mild procedure featuring the use of a Mitsunobu reaction. This approach has been incorporated in a powerful method for site-specific *N*-alkylation of peptides as is exemplified by an *N*-ethyl scan of Leu-enkephalin. Under present investigation is the application of *N*-alkylation in conformational constraints of peptides.

ACKNOWLEDGMENTS: These investigations were supported by The Netherlands Foundation for Chemical Research (SON) with financial aid from The Netherlands Technology foundation. We thank Dr. C. Versluis for recording and interpretation of the mass spectra of the Leu-Enkephalin derivatives, Dr. Ir. J.A.W. Kruijtzer for helpful discussions and suggestions.

REFERENCES AND NOTES

1. See *e.g.* Adang, A.E.P.; Hermkens, P.H.H.; Linders, J.T.M.; Ottenheijm, H.C.J.; van Staveren, C.J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 63.
2. For a reviews see *e.g.* Giannis, A.; Kolter, T.; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244; Gante, J. *ibid.* **1994**, *33*, 1699.
3. (a) Simon, R.J.; Kania, R.S.; Zuckermann, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K.; Spellmeyer, D.C.; Tan, R.; Frankel, A.D.; Santi, D.V.; Cohen, F.E.; Bartlett, P.A. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 9367; (b) Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 543.
4. Miller, S.M.; Simon, R.J.; Ng, S.; Zuckermann, R.N.; Kerr, J.M.; Moss, W.H. *Drug Develop. Res.* **1995**, *35*, 20.
5. (a) Kruijtzer, J.A.W.; Liskamp, R.M.J. *Tetrahedron Lett.* **1995**, *36*, 6969; (b) Kruijtzer, J.A.W. *Thesis* **1996** Utrecht University, The Netherlands; Kruijtzer, J.A.W.; Liskamp, R.M.J. *manuscript in preparation.*
6. Pietzonka T.; Seebach, D. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1481.
7. Miller, S.C.; Scanlan, T.S. *J. Am. Chem..Soc.* **1997**, *119*, 2301.
8. See *e.g.* Abdel-Magid, A.F; Carson, K.G.; Harrisk, B.D.; Maryanoff, C.A.; Shah, R.D. *J. Org. Chem.* **1996**, *61*, 3849.
9. (a) Gordon, D.W.; Steele, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 47; (b) Szardenings, A.K.; Burkoth, T.S.; Look, G.C.; Campbell, D.A. *J. Org. Chem.* **1996**, *61*, 6720; (c) Nefzi, A.; Ostresh, J.M.; Houghten, R.A. *Tetrahedron Lett.* **1997**, *38*, 4943.
10. Wisniewski K.; Kolodziejczk A.S. *Tetrahedron Lett.* **1997**, *38*, 483.
11. Tsunoda, T.; Otsuka, J.; Yamamiya, Y.; Itô, S. *Chem Lett.* **1994**, 539
12. Fukuyama, T.; Jow, C.-K.; Chueng, M. *Tetrahedron Lett.* **1995**, *36*, 6373.
13. The yield of **4b** was low due to purification problems.
14. Abbreviations: PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; DIC, *N,N'*-diisopropylcarbodiimide; BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; TCFH, tetramethylchloroformamidinium hexafluorophosphate; CIP, 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate; PyClU chloro-*N,N,N'',N''*-bis(tetramethylene)formamidinium hexafluorophosphate; BOP-Cl, *N,N*-bis(2-oxo-3-oxazolidinyl)phoshinic chloride; HOAt, 1-hydroxy-7-azabenzotriazole; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.
15. Sieber, P. *Tetrahedron Lett.* **1997**, *28*, 6147.
16. Typical procedure for the preparation of Boc-Ser(Bn)-Ala-(NEt)Leu-OMe **10c**: Fmoc was removed from **6** by treatment with 20% piperidine in NMP for 0.5 h, followed by washing with DMF. The free amine was treated with 5 eq. *o*-NBS-Cl, 5 eq. DiPEA in DMF for 0.5 h, followed by washing with DMF and CH_2Cl_2, respectively. The sulfonamide was treated with 5 eq. PPh_3, 5 eq. EtOH, 5 eq. DEAD in CH_2Cl_2 for 0.5 h, followed by an extensive washing with DMF. The *o*-NBS group was removed by 3 eq KO*t*-Bu, 5 eq. PhSH in DMF or 3 eq DBU, 5 eq. $HSCH_2CH_2OH$ in DMF for 0.5 h, followed by washing with 25% HOAc/DMF, DMF, 5% DiPEA/DMF and DMF, respectively. The resulting secondary amine was treated with 3 eq. Boc-Ala-OH, 3 eq. PyBroP, 3 eq. HOAt, 9 eq. Dipea in DMF for 16 h, followed by washing with DMF and CH_2Cl_2. The Boc-group was removed with 4 N HCl in Dioxane for 0.5 h, followed by washing with CH_2Cl_2 and DMF, respectively. The free amine was treated with 3 eq. Boc-Ser(Bn)-OH, 3 eq. BOP, 6 eq. Dipea in DMF for 1 h, followed by washing with resp. DMF and CH_2Cl_2. The peptides were cleaved from the resin by treatment with catalytic amount of NaCN in MeOH for 16 h and purified by silicagel column chromatography.